DISCUSSION

The overall discussions on the findings of present the study are presented in this Chapter. This Chapter critically discusses the results of the in vivo experiments on *Heteropneustes fossilis* in the laboratory conditions. Analysis of polluted water samples for the presence of estrogenic heavy metals and estrogenic chemicals are also discussed weighing against the other relevant research works done by different researchers in similar fields.

As evidence of the threat of endocrine disrupting chemicals (EDCs) is mounting, there is a need to revise our research efforts. EDCs present a novel challenge to the evolved endocrine processes of both humans and wildlife species. The studies described in the thesis have highlighted the potential weak estrogenic activity as well as toxic properties of known xenoestrogen Bisphenol A. The initial studies were undertaken to investigate the estrogenic activity of BPA by evaluating the morphometric characteristics, which are viewed as general indicators of endocrine disruption. In addition to the estrogenic activity toxic study of BPA was also undertaken. It was followed by histopathological study, ultrstructural study, sperm count studies. To find out the genotoxicity of BPA, micronucleus assay was followed together with abnormal erythrocytic count study to ascertain cytotoxicity. Our results supported the hypothesis that exposure to BPA at low doses can have potential estrogenic and toxic effects. Human exposure to BPA due to its widespread use, along with reproductive and developmental effects reported in animal studies has generated considerable attention on this chemical in recent years. Keeping this in mind a series of experiments were conducted in the present study to assess the effects of BPA on fish *Heteropneustes fossilis*.  

145
5.1. Acute toxicity of Bisphenol A

Bisphenol A has low acute toxicity toward aquatic organisms. In the present study 96-h LC_{50} values of Bisphenol A was 7ppm (7mg/l). Acute toxicity levels for BPA, defined as the concentration at which half of the organisms survive (LC_{50} values), have been measured in a variety of aquatic organisms, including freshwater and saltwater algae, invertebrates (daphnids and mysid shrimp) and fish. LC_{50} values of BPA range from 1000 to 20,000 µg/l (Staples et al., 1998; Staples et al., 2002). Another study suggested that the LC_{50} levels for fish range from 1.1 to 10mg/l (Alexander et al., 1988). Algal EC_{50} was found to be 1000 µg/l (Staples et al., 1998). Exposure of brown hydra (Hydra littoralis) to 2-4 mg/l BPA had toxic effects on both sexual and asexual reproduction (Fukuhori et al., 2005). A similar study found that the 96 hour LC_{50} value for BPA was 6.9 mg/l for pink hydra (Hydra vulgaris) (Pascoe et al., 2002). Yokota et al. (2000) estimated the 96-h LC_{50} values of BPA to be 13,900 µg/l for 24-h-old larvae of medaka (Oryzias latipes) and 13,000 µg/l for adult medaka. According to current U.S. Environmental Protection Agency standard evaluation procedures, bisphenol A was moderately to slightly toxic to the fish and invertebrates tested, with LC_{50} or EC_{50} values of from 1.1 to 10 mg/l. Thus the present acute toxicity study in Heteropneustes fossilis is in accordance with studies going across the world.

5.2. Effect of BPA on Relative liver weight

A biomarker of estrogen exposure is liver size and weight (Lye et al., 1997). Abundant data are available on the toxicity of BPA (Chen et al., 2002). Somatic indices measure the overall condition of the fish (West, 1990). The results of the present study showed a gradual increase in liver size and weight, after being exposed to E_2 and BPA (0.8 ppm, 1 ppm and 5 ppm) after a period of 7 days and 14 days (Chapter 4, Fig. 4.2). No significant effect was seen in the acute study of 24 hours and 48 hours (Chapter 4, Fig. 4.3). In similar studies fishes exposed to effluent mixtures from sewage plants and paper mills have resulted in an increased liver size (Folmar et al., 1996; Jobling et al., 1996; Lye et al., 1997; Purdom et al., 1994). According to another study, Cyprinus carpio when exposed to 0.001 mg/l of endosulfan for 15 days a significant increase in
Liver weight was seen (Salvo et al., 2008). Thus the increase of liver size due to BPA administration is in accordance with similar studies of other authors.

A major function of the liver during ovarian recrudescence is the synthesis of vitellogenin (Kime, 1998). The liver in the mature female teleost, is the primary target tissue of 17β-estradiol. Binding of 17β-estradiol to the estrogen receptor in hepatocytes induces the synthesis of vitellogenin (VTG), a glycolipophosphoprotein that serves as the precursor for yolk proteins (Lazier and MacKay, 1993). Although both male and female fish express the ER, only the female is capable of synthesising the steroid 17β-estradiol, which is produced in the ovaries. Male fish have fewer receptors and in the brown trout (Salmo trutta) the liver of a sexually mature female contains more than twice as many estrogen-binding sites as that of the male (Pottinger, 1986). Receptors present in male fish are fully functional and capable of binding to EDCs and, hence, VTG synthesis may be induced. In juvenile fish and mature males, exposure to estrogens can induce synthesis of this protein. Most xenestrogens act by a similar mechanism but have less affinity for the ER, thus, less potency than 17β-estradiol (Nimrod and Benson, 1996). Thus the increased liver weight might be due to resultant hepatocyte proliferation due to the accelerated synthesis of vitellogenin, as it has been found that there is a constant protein concentration during such exposures (Van Boheman et al., 1982).

5.3. Effect of BPA on HSI

The Hepatosomatic index (HSI) of the fish has been used as an indicator of environmental risk (Pinkney et al., 2001; Yang and Baumann, 2006) and is a good predictor of adverse health in fish (Adams and McLean, 1985). The HSI is seen to increase in fish that have been exposed to estrogenic compounds (Herman and Kincaid, 1988; Van Boheman et al., 1982; Nimrod and Benson, 1996; Lye et al., 1997; Thorpe et al., 2000; Harries et al., 1997). In the present investigation, HSI showed a slight increase at p<0.05 among the fishes which were treated with BPA. Similar trend of increased HSI was observed in estradiol treatment group. These data appreciably support a number of scientific reports. Herman and Kincaid (1988) reported significantly higher HSI in rainb-
bow trout males when exposed to estradiol in lab experiments. The HSI values of the fish subjected to phenol polluted water also increased (Abdel-Hameid, 2007). Thus considering above mentioned reports, it is suggested that BPA induced increased HSI was possible. According to (Medda et al., 1980 Christensen et al., 1999), Vitellogenin induction in general may result in enhanced liver metabolism leading to an enlargement in the liver and consequently an increased HSI. Thus the increase liver size and as well as HSI due to BPA treatment might be due to accelerated production of vitellogenin.

5.4. Effects of BPA on Testis weight

Although the presence of vitellogenin is the most widely accepted biomarker of estrogenic exposure, there are other physiological and biochemical measurements which are viewed as general indicators of endocrine disruption (Sumpter and Jobling, 1995). In general, inhibition of testis growth in male fish has been reported in connection with exposure to estrogens and can be said as a biomarker of estrogen response (Billard, 1981; Jobling et al., 1996). Studies on rainbow trout (Oncorhynchus mykiss) have shown a correlation between the estrogenic potency of an EDC and the degree of inhibition of testicular growth, with certain chemicals reducing testicular growth by as much as 50% (Billard et al. 1981; Jobling et al., 1996). Additionally, Billard et al. (1981) found that the inhibitory effects varied according to dose, type of steroid, and stage of testicular development at the time of exposure. In the present study with Bisphenol A, testis weight was seen to decrease significantly. No significant effect on testicular weight was marked after 24 hr, 48 hr and 7 days exposure. Results were obtained only after 14 days treatment. 14 day, exposure with BPA showed significant decrease (p<0.05) of testis weight at low (0.8ppm), medium (1ppm) as well as highest dose of 5ppm. E2 treated group also showed significant decrease in testis weight (Chaper 4, Fig. 4.6 and 4.7). Thus BPA in the present scenario had a time dependent effect on testis weight. This result corresponds to the finding of many other authors. In a study by (Gimeno et al., 1998) male carp exposed to 4 - tert pentylyphenol (TPP) resulted in reduced testicular growth.
5.5. Effect of BPA on GSI

Roy, 2011

and correspondingly lower testicular weight. A reduction in testis mass leading to reduced GSI has been described as an estrogenic effect by (Jobling et al., 1996; Cristiansen et al., 1998). A dose dependent atrophy of testis in flounder treated with DDT was reported by (Zaroogian et al., 2001). Thus this result corresponds to the finding of other authors.

The mechanisms underlying inhibition of testicular growth by estrogenic chemicals are not known. It is not clear if the effect is due to disruption via the pituitary-hypothalamic system or due to direct action on the testis itself, but it is possible that these chemicals exert their effects directly on the testis, possibly via the inhibition of androgen synthesis (Jobling et al., 1996). The EDCs therefore may have direct effects on the testis either (i) cytotoxicologically, where the disruption is caused by damage to the cellular integrity or function of gonadal cells in general or (ii) endocrinologically, in which the functions of specific cells are disrupted due to an endocrine malfunction. This could be due to altered pituitary secretions or to alterations in the chain of enzymes within the testes, which lead to synthesis of the testicular hormones (Kime, 1998). It is also possible that estrogenic chemicals inhibit spermatogenesis (sperm production) by acting at one or more levels in the cascade of hormones that regulate development of the testes. For example, estrogens may inhibit GnRH synthesis in the hypothalamus, and/or gonadotropin synthesis in the pituitary gland (Jobling et al., 1996). Thus BPA induced reduction in testis weight is mainly due to its estrogenic cascade of actions and is in broad accordance with the studies of authors working in the similar fields.

5.5. Effects of BPA on GSI

Decreased gonadosomatic index, GSI (the ratio of gonad weight to whole body weight), in fish exposed to xenobiotics has been reported by many authors (Jobling et al., 1996; Billard et al., 1981). Gonads of developing male animals are very sensitive to estrogens. This was clearly demonstrated by the complete inhibition of gonadal development when maturing male salmonids were exposed to 17β-estradiol via their diet (Billard, 1981). Although mature fish may also be affected, as in viviparous guppies

149
5.5. Effect of BPA on GSI

*Poecilia reticulata* blocked spermatogonial mitosis was seen in the testis structure after exposure to octyphenol and 17β-estradiol (Kinnberg *et al.*, 2003).

BPA had effects on the GSI of the experimental animals in the present study. In present investigation, GSI showed a decreasing trend at p<0.05 among the fishes which were treated with BPA (Chapter 4, Table 4.2). Similar trend of decreased GSI was observed in 17β-estradiol treatment group (Chapter 4, Table 4.2). These data are in broad accordance with a number of scientific reports. Sohoni *et al.* (2001) investigated the long term reproductive effects of BPA and found reduction in GSI in fathead minnows (*Pimphales promelas*). Monosson *et al.* (1994) injected groups of female adult white perch with coplanar PCB and found significant reduction in GSI. Similarly reduction in GSI in male medaka was observed after treatment with Bisphenol A metabolite MBP (4-methyl-2,4-bis (4hydroxyphenyl) pent-1-ene (Ishibashi *et al.*, 2005). Kang *et al.*, (2002) has observed significant decrease in GSI after exposing medaka to 17β-estradiol and 4-nonylphenol. Moreover when summer flounder (*Paralichthys dentatus*) was treated with Octylphenol reduction in GSI was observed. Male fishes (*Poecilia reticulata*) exhibited decreased GSI when treated with Nonyl phenol (Cardinalli *et al.*, 2004).

The reduction of testis growth and hence GSI is usual in individuals treated with sex steroids from opposite sex (Billard *et al.*, 1986) as well as in fish exposed to contaminants with estrogenic activity (Jobling *et al.*, 1996; Kinnberg *et al.*, 2000). GSI decreases have also been reported in adult male trout and carp exposed to estrogens either in water or via food (Komen *et al.*, 1989). Thus, the present data on the effects of BPA on gonad weight and GSI showed a strong agreement with the above mentioned reports. GSI is often applied as an endpoint of endocrine disruption because a reduced GSI usually points to a reduction in gonad mass.
5.6. Effects of BPA on Histoarchitecture of Liver and Testis of *Heteropneustes fossilis*

5.6.1. Effects of BPA on Liver

The liver of fish can be considered a target organ to pollutants and it exhibits the effects of a variety of environmental pollutants (Hinton *et al.*, 1992). Alterations in its structure can be significant in the evaluation of fish health (Myers *et al.*, 1998). The histopathological studies on the liver of *H.fossilis* in the control group showed the presence of distinct hepatocytes, the basic functional unit and exocrine pancreatic tissue that surrounded the portal veins. Hepatocytes appeared as polyhedral cells with centrally located nucleus and seemed to be arranged radially in the form of distinct cords around hepatic vein. Sinusoids were distinct which separated the adjacent hepatic cords. Liver treated with BPA after 24 hr showed certain degenerative changes. Fatty infiltration (steatosis), destruction of cellular structures was evident. The borders of the hepatocytes were obscure and cell degeneration was marked. The effect on liver was more pronounced during longer exposure schedules of 48 hr, 7 and 14 days. Disrupted parenchymal architecture and necrosis, pycknotic nuclei, destruction of cellular structures and extensive hepatic hemorrhage together with necrosis was noticeable. This observation was similar to the study of (Murmu and Srivastava, 2010). They observed hypertrophied hepatic nuclei, characterized by pycknotic nuclei in hepatic cells and necrosis after 15 days of treatment with BPA in *C. mrigala*. It was also observed that the intensity of damage was more in longer days treated groups *i.e.*, changes in liver were more prominent in longer duration.

The fibrosis, steatosis, and necrosis; the hepatic tissue changes, were similar to those reported from fish caught in contaminated water or exposed ones to various chemicals in laboratory conditions (Brand *et al.*, 2001; Koehler, 2004; Olojo *et al.*, 2005; Camargo and Martinez, 2007; Wahbi and El-Greisy, 2007; Aniladevi *et al.*, 2008). Fibrosis and local blood congestions in the liver sinusoids of the flounder, *Platichthys flesus*, the ruffe, *Gymnocephalus cernua*, and the smelt, *Osmerus eperlanus*, were
reported as a consequence of pollution by Peters et al. (1987). Radhaiah and Jayantha (1992) reported moderate cytoplasmic degeneration in hepatocytes, formation of vacuoles, rupture in blood vessels, and pyknotic nuclei in the liver of *Tilapia mossambica* exposed to fenvalerate. Likewise Tilak et al. (2005) observed the same changes in liver of *Catla catla* exposed to chlorpyrifos. Gupta and Guha (2006) also observed related changes in the liver after microcystin exposure in *H. fossilis*.

BPA, an environmental oestrogenic chemical, is glucuronidated in rat liver microsomes and furthermore, that the isoform UGT2B1 is responsible for their glucuronidation (Yokota et al., 1999). Knaak and Sullivan (1966) reported that 28% of BPA was excreted in urine, primarily as the glucuronide. Glucuronidation is a major detoxification pathway in all vertebrates, whereas it is rare in invertebrates (Dutton, 1980). BPA has been reported to be hydroxylated *in vivo* (Knaak and Sullivan, 1966) and to bind to DNA (Atkinson and Roy, 1995). It has also been reported in a similar study that ROS (Reactive oxygen species) were induced in the liver of mouse when BPA was administered throughout the fetal life and during infancy (Chitra et al., 2002, Kabuto et al., 2003; 2004). Another work has demonstrated that BPA generate ROS and inflict oxidative stress by affecting the redox status of the exposed organ (Atkinson and Roy, 1995, Hasselberg et al., 2004, Korkmaz et al., 2010). Furthermore it has been shown by Sax (1985) and Suarez et al. (2000) that absorption of large amount of BPA through skin causes extensive damage to the liver. BPA has shown to cause the formation of multinucleated giant cells in rat liver hepatocytes (National Toxicology Program, 1982; Nagakawa and Tayama 2000). Thus, considering above reports with the results of the present study, it can be concluded that the differential effects observed in the histoarchitecture of liver tissues following BPA exposure may induce long-term changes in the adult fish.

### 5.6.2. Effects of BPA on Testis

It is well known that certain environmental chemicals may affect male and female reproductive performance (Fry and Toone, 1981; Lye et al., 1997). Male fish exposed to
such compounds may exhibit a range of reproductive problems including constrained or disrupted testicular structure. Additionally the changes in the histology of the testis are an indication of the disturbances to the HPG axis (Wester et al., 2002).

The histopathological studies on the testis of *H. fossilis* in the control and solvent control group in the present study showed the presence of numerous lobules which are separated by a thin layer of connective tissue. These lobular compartments are the seminiferous tubules which consisted of Sertoli cells and germ cells. Spermatogonia are located alongside the entire length of the tubule. Acute study of 24 hr and 48 hr showed no degenerative changes in the testis and were more or less similar to controls. Seminiferous tubules filled with spermatozoa were observed. Sertoli cells were also seen. Degenerative changes were marked after a longer duration exposure of 7 and 14 days respectively. Disintegration of testicular structure, shrunken seminiferous tubules and lumen with less spermatozoa and very less or absence of Sertoli cells was observed. Moreover the lobular structure of testis was lost and necrotic testis was observed at the higher doses. The effects of both BPA and 17β-estradiol were enhanced in the longer durations and depended on both concentration and duration of study. This correlates with study of Janecki et al. (1992). They investigated the effects of cadmium chloride on Sertoli cell function and reported that toxic effects depended on the concentration and duration of the study. In a similar study effect of estrogenic chemicals such as 4 tert octylphenol and BPA on sexually mature male guppies demonstrated the ability of these contaminants to adversely affect testis structure (Haubruge et al., 2000; Kinnberg et al., 2000). E\textsubscript{2} also has similar effects on testis of mature guppies (Kinnberg et al., 2000). Recently it was shown that exposure to high concentration of nonylphenol and 17β-estradiol resulted in adverse effects on the testis structure of mature male guppies (Kinnberg et al., 2000). The histological examination of the testis of *Z. viviparous* revealed severe effects on the testicular structure of the seminiferous tubules in response to high dose of nonylphenol as well as 17β-estradiol (Christiansen et al., 1998). It was also observed that histological alterations were also obtained at low doses (Christiansen et al., 1998). Further study revealed the reduction in number of spermatozoa in the epididymis in mice treated neonatally with BPA (Nakahashi et al., 2001). Thus the
present study is in broad accordance with other studies and that BPA probably induces disrupted testicular structure.

In order to elucidate the mechanism of BPA induced testicular dysfunction several hypotheses have been advocated on possible mechanism of action of the reproductive toxicants. A possible explanation is a direct effect on the Sertoli cells that may result in such changes. Various toxicants as well as endocrine disruptors including BPA are known to affect Sertoli cells (Monsees et al., 2000). Such a direct effect on Sertoli cells is supported by the fact that estrogen receptors are found in Sertoli cells in mammals (Nakhla et al., 1984). Preliminary evidence suggests that they are also found in the dogfish *Squalus acanthias* (Dubois and Callard, 1989). Besides in the eel *Anguilla anguilla* ethynylestradiol inhibits development or causes degeneration of the Sertoli cells (Colombo and Grandi, 1995). Estrogenic chemicals may also exert their effects directly on the testis via inhibition of androgen synthesis (Trudeau et al., 1993; Jobling et al., 1996). They may also act indirectly via the hypothalamo-pituitary-gonad axis, inhibiting the synthesis and secretion of gonadotropin releasing hormones or gonadotropins (Jobling et al., 1996). Some authors have also reported that BPA generate ROS (reactive oxygen species) and inflict oxidative stress by affecting the redox status of the exposed organ (Atkinson and Roy, 1995; Hasselberg et al., 2004) leading to such alterations. Thus the present study corroborates to the finding of other authors.

### 5.7. Effects of BPA on Liver (SEM studies)

Sperm morphology could provide a sensitive and accurate bioindicator of aquatic pollution (Kime, 1996). BPA induced hepatic toxicity is well suited to study by SEM because it involves alterations to cell surface morphology and the relationship between parenchymal and sinusoidal cells. In the present study, after treatment with BPA for 7 days characteristic swelling of liver cells were visible and sinusoids were congested. In the 17β-estradiol (positive control) group hepatocytes had lost their roundish contour and had increased in size. At all doses of BPA the liver hepatocytes had swelled and sinusoids
were congested. Occasional blebs were also seen. After 14 days of treatment higher deformities were marked. The cell boundary of hepatocytes was distorted. Characteristic swelling of cells was more evident and was accompanied with vacuoles. In the 17β-estradiol group also the hepatocytes had lost their roundish contour and increased in size. At all doses of BPA the liver hepatocytes had swelled and sinusoids were not visible. Extrusion of cellular contents was noticeable.

Paradoxically, toxic exposure can result in accumulations of fat or glycogen in the liver. This type of dichotomy (i.e., glycogen accumulation versus depletion as a result of toxicity) was evident in experiments in rainbow trout (Seinen et al., 1981), Japanese medaka Orezias latipes (Wester et al., 1988), guppies Poecilia reticulata (Wester and Canton, 1987), and rats (Krajnc et al., 1984) when they were exposed to organotins such as bis(tri-n-butyltin)oxide (TBTO), di-n-butyltindichloride (DBTC), and tri-n-butyltin chloride (TBTC). Lipid or glycogen vacuolization can cause an increase in the size of hepatocytes (Wolf and Wolfe, 2005) However, Hinton et al. (1992) identified 3 additional potential causes of hepatocellular enlargement: organelle proliferation (hypertrophy); the failure of sublethally-injured hepatocytes to mitotically divide (megalocytosis); and vacuolar swelling of the endoplasmic reticulum cisternae (hydropic degeneration). Hepatocyte hypertrophy was observed in rainbow trout that were simultaneously exposed to endosulfan and disulfoton (Arnold et al., 1995). It was observed that damage from toxic or immunologic insult may cause hepatocytes to take on a swollen edematous appearance which is generally known as ballooning degeneration. Thus BPA which is known to inflict immunologic insult as well as toxic damage can also be the reason for the increased hepatocyte size.

Congestion of sinusoids was another feature observed in SEM studies. Congestion implies increased numbers of RBCs within the vasculature in the congested liver erythrocytes both within sinusoids and Disse space. Liver congestion is associated with damage caused by many toxic agents. The changes caused by these compounds are thought to arise as a consequence of binding to the microfilament component of the cytoskeleton at cell margins. Walker et al. (1983) found similar results after acetami-
5.8. Effects of BPA on Testis (SEM studies)

- nophen treatment. Their results indicated that congestion if prolonged could result in impaired liver circulation and consequent hypoxic injury. Furthermore congestion develops before the appearance of necrosis and is an important and distinctive aspect of hepatotoxicity (Walker et al., 1983). Additionally when the liver tissue was viewed under SEM blebs were observed. These blebs could be caused by low oxygen conditions within the liver tissue as noted by Lemasters and Stemkowski, (1983). They concluded that the blebs could be hepatocyte microvilli that swell under conditions of hypoxia.

Thus in the present study BPA might cause cellular hypertrophy which might lead to congestion of sinusoids and consequent hypoxic injury to the liver.

5.8. Effects of BPA on Testis (SEM studies)

Scanning electron microscopy, due to its large depth of field and high resolving power appears to be important in studying surface ultra structural morphology and organization of the spermatozoa. Many authors use this versatile tool with success in understanding the ultra structural morphology of sperm and other structures either in normal fish or in fish experiencing some kinds of stress (Dey et al., 2009 a, b; Psenicka et al., 2007). SEM has been proved to be versatile in assessing the effect of environmental stress, pollution, and toxic chemicals on surface morphology of various tissues in a number of animals (Dey et al., 2001, 2005). In the present study the sperm head in the estradiol and BPA treated fishes exhibited abnormal shape. Some fish revealed remarkable distortion of the sperm-head where some were found to be clumped. Further, the present study demonstrated occasional occurrence of sperm agglutinations in the higher doses of BPA groups. The spermatozoa were agglutinated in a head-to-head manner, which invariably makes them fail to fertilize the egg as reported by Mochida and Takahashi. (1993). It seems likely that the sperm agglutination results from an influence of autologous IgM, which comes to penetrate the efferent duct as autoimmune responses in the testis progress. Lou and Takahashi. (1991) showed that in the Nile tilapia, autoantigens existed locally on the head of spermatozoa as determined by immunecytochemical method.
using anti-sperm autoantibody purified from the serum of immunized fish. In this context, it is also to be noted that normal sperm-length is extremely important for sperm motility, because sperm-length parameter is correlated positively with ATP, energy charge and fertilization success (Vladic et al., 2002). The movement of spermatozoa in most of the fish is controlled by energetic and cytoplasmic ionic conditions which are responsible for marked changes in cell morphology (Dreanno et al., 1999). This supports the observations on the absence of well-differentiated acrosomes, mid-piece and the existence of very short sperm-tails in BPA treated *H. fossilis*. The observations on the clumping of spermatozoa, poorly developed sperm-tails and distortion of sperm-head in some cases in *H. fossilis* treated with BPA suggests that sperm motility is adversely affected. This was confirmed through the observations in control fish which exhibited well-differentiated head and sperm-tail of normal length. As high motility of sperm is a prerequisite for fertilization and since it correlates strongly with fertilization successes (Rurangwa et al., 2004), the present observation on poorly developed sperm tail in BPA treated *H. fossilis* is certainly one of the major causes for impairment of reproductive function in the fish leading to its gradual population decline. Thus the present observation on shortening of the sperm tail as well as its abnormal structural features in some of the sperm appears to be relevant to the poor sperm count studies (presented later) which may thus lead to failure in fertilization.

**5.9. Effects of BPA on Liver (TEM studies)**

Some chemicals result in the structural and functional adaptive responses in the liver. Although a number of adaptive responses of the hepatocyte are of a metabolic nature, morphologic changes frequently may be noted microscopically and on some occasions, grossly. In the present study BPA exposure produced cytotoxic changes in *Heteropneustes* liver with hepatocytes as, by far, the major cellular target. As observed by TEM differences were noted in the cellular organelles. Alteration in the cell surface reflects hormonal imbalance or other substances that interact with the cell.
Another important finding was the alteration in the activity of the hepatocellular organelles. Such alterations are designated as Hepatocellular Adaptive Responses.

The TEM analysis showed the nucleus as enlarged and de-shaped. The nucleolus showed some contour alteration. In the treated group the nuclear contour was difficult to be clearly observed, suggesting loss of the membrane integrity. Moreover nuclear membrane was absent at some points. Absence of nuclear membrane is a serious problem because if there is no nuclear membrane the cell would have no organization of the genetic material. It was observed that abnormal nuclear morphology is a cell death pathway (Weber et al., 2004) leading to necrosis. In the present study the necrosis was further demonstrated by the cellular swelling, and the loss of nuclear membrane. Other than nuclear distortions, some mitochondria alterations were also observed in the BPA and 17 β-estradiol treated groups. The mitochondrial cristae presented degenerative alterations, fragmented in aspect or were even absent. This proves the toxic ability of BPA to inflict stress on the cellular organelles. Acute accumulation of ceramids, might directly or indirectly, deeply affected the mitochondrial functions (Tomassini and Testi, 2002). Mitochondrial toxicity in pregnant rats and their fetuses were observed after cisplatin treatment by Gerschenson et al. (2002). It was found that cisplatin forms DNA adducts and causes mitochondrial changes. Similar might be the case of BPA. It is known that BPA produce DNA adducts and generate ROS and inflict oxidative stress by affecting the redox status of the exposed organ (Atkinson and Roy, 1995; Hasselberg et al., 2004). The mitochondrial defects observed might be because of this adduct formation and ROS.

In the treated groups RER was highly altered with some areas presenting a high concentration of expanded cisterns in concentric circles. It was also found that the RER volume was greatly increased. These features indicated the occurrence of an intense protein synthesis as a possible response of the cell to recover its homeostasis through lysosomal enzyme and membrane protein production (Scaff and Scussel, 2008). This increase is mainly due to additional production of ER, part of the ER increase may be related to impaired membrane catabolism. This proves pathological condition of the cell. Thus it clearly indicates that BPA causes stress to the RER and to recover from this stress increased amount of protein synthesis is required. Thus BPA induces abnormal
morphologically altered cellular organelle structure as well as function. As very less work is done in this field more work will help to better understand the mechanisms involved.

5.10. Effects of BPA on Testis (TEM studies)

Sperm morphology could provide a sensitive and accurate bioindicator of aquatic pollution. It is well known that normal structural features will ensure the proper functioning of the sperm which is directly related to the success in fertilization. The detail understanding of the cellular features of spermatozoa was possible through extensive studies involving TEM (Gusmao-Pomoiani et al., 2009). Several authors have used electron microscopy for studying the ultra structure of spermatozoa from different viewpoints. It is to be noted in this context that despite a large number of published works in the existing literature about sperm ultra structure, very few reports exist on the same topic with reference to effects of BPA. The basic task of the spermatozoon head is to transfer genetic material localized in the nucleoplasma to the egg. Hence, an optimal shape and size of sperm head is a prerequisite for proper penetration of spermatozoon through the micropyle of egg (Psenicka et al., 2007). In this context, the membrane dislocation in some parts of the sperm-head and its outward expansion in treated fishes with BPA in the current study have definitely caused deformity in shape of the spermatozoon head. This transformation is likely to have adverse effect on the primary function of the sperm-head. Further, an essential feature of every cell is the presence of membranes that define the cell boundary and various internal components of cells. Cell membrane serves as a locus of specific functions and possesses transport proteins that facilitate and regulate the movement of substances into and out of the cell and its compartments. On that consideration, the dislocation of sperm head membrane, and breakage of sperm tail membrane at places as observed in the current study are likely to cause adverse effects on functioning of the sperm of the fish treated with BPA. Pertinent here, is to mention that sperm plasma membrane is reported to play a very active role in sperm fertilization capacity and in spermatozoon-oocyte cross-talk (Lenzi et al., 1996).
5.10. Effects of BPA on Testis (TEM studies) Roy, 2011

Poor differentiation of head and neck of the sperm, and abnormalities in the shape of sperm head in the fishes treated with BPA, revealed in the current study suggests that BPA has serious adverse effects on the male reproductive unit of the fish.

In addition, the transverse section of the sperm tail show distortion and breakage at places along with disturbances in micro-tubular assembly. Defects in the structure of axoneme, in the form of missing peripheral microtubules or, more commonly, missing central microtubules were evident. In some affected specimens, the mitochondria showed signs of disintegration and disorganization. Similarly, Au et al. (2000) also reported disorganization of mitochondrial membranes and cristae and disrupted ATP supply for sperm movement in spermatozoa of mussel and sea urchin after cadmium and phenol treatment. The distortion of mitochondria in the lateral lobes of neck in many of the sperm of fish suggests disturbances in energy release required for sperm movement of the fish. The intense vacuolization in sperm-head in the region enclosed by the dislocated and extended membrane of fish treated with BPA is highly significant in view of the fact that a close relation between vacuolization and apoptosis has been reported by some authors (Gonzalez-Polo et al., 2005).

Effects of these environmentally relevant concentrations of BPA on sperm were similar to those observed in fishes from polluted waters of Umiam lake, (Massar et al., 2011). It has been suggested that the increasing incidence of male reproductive anomalies in fish and other animals may be the result of environmental pollution by toxic chemicals (Toppari et al., 1995). Heavy metals in particular, are known to be associated with altered steroid levels and hindered gonadal development in a variety of fish species (Joy and Kirubagar, 1989; Wester and Canton, 1992). Mercury is reported to damage sperm resulting in decreased sperm motility probably by interfering with flagella function (Mottet and Landolt, 1987). A significant decrease in motility of cat fish sperm exposed to cadmium and zinc has also been reported (Kime et al., 1996). Furthermore, Gill et al. (2002) described sperm abnormalities in head and tail, e.g. big heads and fuzzy tails, in flounders taken from the lower Tyne estuary in northeast England, a heavily impacted site known to contain endocrine disrupting chemicals. Thus these fine structural abnormalities
are bound to cause physiological and functional disturbances in the sperm, leading to reproductive inefficiency.

Despite the fact that studies on the effect of environmental pollution on fish sperm have been carried out with a number of different approaches, ultrastructural aspect of the same which explains the effect with better precision are lacking in the existing literature except a few. The present study is thus relevant in understanding the adverse effect of environmental toxicant BPA on fish sperm ultrastructure, which explains the possible functional problems. The observations made in the current study suggests that SEM and TEM should be included as essential components in studies on environmental pollution affecting vital tissues of fish, so that the deleterious effects on the functional physiology can be understood. Besides this, information on ultra-structure can help the investigators to use other approaches to address the problem.

5.11. Effects of BPA on Sperm count

The testicular sperm counts and daily sperm production are important indicators for investigators to detect adverse effects of various factors on spermatogenesis (Ban et al., 1995; Wang et al., 2004; Xu et al., 2006). Exposure to low levels of potentially estrogenic compounds affects the number of sperm produced. Sperm count is a fundamentally important predictor of male fertility and it is therefore important to assess the effects of xenobiotics so that the risk assessments of endangered and commercially important species can be addressed. In humans there is evidence that sperm counts are in decline possibly as a result of exposure to xenobiotics (Carlsen et al., 1992; Sharpe and Shakkebaek, 1993).

The primary finding was that 17β-estradiol and BPA had some deleterious effects on sperms. From the experimental results, it was observed that there was significant reduction in sperm count at all doses of BPA (0.8 ppm, 1 ppm, and 5 ppm) and 0.1 ppm 17β-estradiol compared to the untreated control (tap water) group. Sperm count was
5.11. Effects of BPA on Sperm count

significantly reduced (p<0.001) at all doses after 7 and 14 days treatment. There was significant effects (p<0.05) only at the highest dose of 5ppm after 48 h study. 24 h study had no significant effect on the sperm count. Therefore it is evident that exposure to low levels of BPA disrupts male spermatogenesis and can generate a decline in sperm count. Significant decrease in counts was seen which coincides with the study of Haubrudge et al. (2000) who observed decrease in sperm counts with BPA exposure after 21 days in Poecila reticulata. Some other studies have recorded male reproductive disruption after chronic exposure to xenobiotics or exposure at particular periods in development when individuals may be especially sensitive to hormone tire (Crisp et al., 1998; vom Saal et al., 1998; Lee et al., 1999). In order to explain the mechanism of decreased sperm count several hypothesis have been advocated on possible mechanism of action of the reproductive toxicants.

The reason for the decline of sperm count study might be a disturbance in the production processes rather than decline in the number of active spermatozoa generated from the germ line. Male Japanese medaka showed compromised fertility at elevated levels after 21 days to the xenoestrogenic alkylphenol 4-tert-octylphenol (Gronen et al., 1999). Another possible explanation is a direct effect on the Sertoli cells that may result in such changes. Various toxicants as well as endocrine disruptors including BPA are known to affect Sertoli cells (Monsees et al., 2000). The mechanisms of such effects on sperm production might be disruption in the germ line. Sertoli cells are essential in spermatogenesis (Jegou, 1992) and these cells are directly sensitive to xenobiotics. Recent research shows that octylphenol (a related xenobiotic to BPA) causes Sertoli cell apoptosis within only 24h of exposure (Raychoudhury et al., 1999). This apoptosis could block the nutritional activity of Sertoli cells on maturing spermatids and arrest the release of gametes from the efferent ducts into the testicular canal and into storage in the deferent ducts of the testis (Billard, 1986). This interference with release could lead to a decline in sperm numbers. Such direct and short term Sertoli cell sensitivity to xenobiotic action could be the mechanism by which exposure to BPA leads to significant decline in sperm counts of H.fossilis.
5.12. Effects of BPA on Sperm viability

The quality of sperm is a major factor contributing to successful production of fish larvae (Kime et al., 1996). Although all male vertebrates produce vastly more sperm than eggs, evidence from both fish and mammals suggests that even a small decrease in sperm quality or quantity can decrease males fertilizing ability. Viability of sperm in fish as in other vertebrates is dependent on the correct hormonal and nutritional environment during their development within the testis (Kime et al., 1996). In the present study analysis of the percentage of dead sperms after 7 days of exposure to different doses of BPA exhibited significant rise (P<0.001) when compared to untreated control (tap water) group. Similarly there was steep decline in percentage of live cells after 14 days of exposure. Similar results were obtained by Oyeyemi et al. (2009) in Dwarf rams treated with Euphorbia hirta, a plant toxin. In another study Rao et al. (1982) exposed 6-month-old male New Zealand white rabbits to 0, 0.1, 1 or 10 ppm DBCP 1,2-Dibromo-3-chloropropane,(a nematocide) vapors (adjusted for 97.3% purity), 6 hours/day, 5 days/, for 14 weeks. The percentage of live sperm in the semen of the rabbits exposed to 10 ppm DBCP was also significantly decreased compared with control values. Rabbits exposed to 1 ppm DBCP, but not those exposed to 0.1 ppm, exhibited significant decreases in the percentage of live sperm during weeks 6, 12 and 13. El-Aziz et al. (1994) also reported that male rats, administered deltamethrin had significantly reduced live cell percentage, relative to controls. Reduced sperm viability effectively reduces the ability of that male fish to fertilize eggs. Ratios of 1,500 sperm per egg are needed for good fertilization rates in the catfish Clarias batrachus (Rurangwa et al., 1998). It has been suggested that male fish in the wild closely control the sperm/egg ratio to achieve the minimum for full fertilization (Warner, 1997).

The possible mechanism of action of BPA in inducing more dead sperms is many. It is seen that heavy metal arsenic (also an endocrine disruptor) causes lipid peroxidation by generation of reactive oxygen species (ROS). (Xu et al., 2008; Osbaldo et al., 1995). Evidence suggests that arsenic induces free radical formation and thus the generated ROS
react with the polyunsaturated fatty acid (PUFA) rich spermatozoa, specially the mid spermatozoa and results in peroxidation which finally leads to destruction in spermatozoa causing decreased viability (Sarkar et al., 2003). Bisphenol A (BPA), is also known for oxidative stress-inducing effect (Rashid et al., 2009). The probable cause of the increase of dead sperm by BPA might be due to the formation of ROS by BPA (Atkinson and Roy, 1995; Chitra et al., 2003). The action of BPA might be of a similar fashion. BPA also caused lipid peroxidation (LPO) and decrease in reduced glutathione (GSH) content of mitochondria (Anjum et al., 2011). Moreover the SEM and TEM results presented earlier (Chapter 4) on the disturbed morphological features further supports the cause of reduction of viable sperms.

5.13. Effect of BPA on frequency of Micronucleus

The micronucleus (MN) test is the most applied technique to evaluate chromosomal damage in different organisms (Anderson et al 1994, Bolognesi et al., 1999, 2004; Campana et al., 2003; Al-Sabti and Metcalfe, 1995) and fish provide a relevant model for evaluation of aquatic genotoxicity (Hayashi et al., 1998). The simplicity and the readiness to be applied to a variety of cell types either in vitro or in vivo made it a versatile tool that contributed to a large extent in our understanding of key toxicological issues related to genotoxins and their effects at cellular and organism levels (Elhajouji et al., 2010). The experimental results in the present study, it was found that micronucleus was induced by BPA when compared to the untreated control (tap water) group. MN was induced significantly at lowest dose (0.8 ppm) and at the highest dose (5 ppm) after 7 day’s treatment and at all doses after 14 days. Similar was the result trend of 17β-estradiol. No significant effect of the said chemicals was observed after 24 and 48 h study.

Pfeiffer et al. (1997) observed that BPA, other than being estrogenic can act as aneuploidogens. Micronucleus was induced by BPA in a study in Atlantic Cod (Gadus morhua) and turbot (Scophthalmus maximus) (Bardiene et al., 2005). Moreover an increase in the frequency of micronucleated cells has been demonstrated by BPA in bone marrow cells of mice (Gudi et al., 1992), in Chinese hamster cells (Pfeiffer et al., 1997), in
5.14. Effects of BPA on frequency of morphologically altered erythrocyte

Human lymphocytes (Suarez et al., 2000) and reticulocytes of mice (Masuda et al., 2005). In an additional study conducted by Indian Council of Medical Research (ICMR), highly significant rise in micronucleated cells were observed with various doses of BPA after 6 days treatment in Adult Holtzman male and female rats. It is known that chromosome breakage and dysfunction of the mitotic apparatus are two basic phenomena leading to the development of micronuclei in mitotic cells (Norpa and Falck, 2003). Thus the increase in micronuclei confirms the genotoxic properties of BPA.

Further recent studies by Johnson and Parry (2008) have demonstrated that BPA induce micronucleus and modify the functioning of the microtubule organizing centre of the mitotic spindle of cultured mammalian cells in a dose dependent manner. Interestingly BPA has the ability to inhibit MT assembly and can cause micronuclei in V79 cells (Pfeiffer et al., 1997). It has also been observed that BPA appears to alter the conformation of the tubulin molecule irreversibly and exhibited potential thresholds for aneugenic activity through disruption of the mitotic spindle. These findings suggest that abnormalities in cell division owing to effects on the mitotic apparatus through tubulin interaction constitute the underlying mechanism of the various toxic effects of BPA (Ochi, 1999). Thus the present study corroborates with studies of other scientist groups.

5.14. Effect of BPA on frequency of morphologically altered erythrocyte

Results of the present study indicate that BPA provoked an alteration of fish erythrocyte morphology from the normal elliptical shape to other forms such as echinocytes, poikilocytes, anisocytes, spherocytes and ovalocytes. From the experimental results, it was seen that morphologically altered erythrocytes was induced by all doses of BPA (0.8ppm, 1ppm and 5ppm) when compared to the untreated control (tap water) group at all the exposure programs. Effects of these environmentally relevant concentrations of BPA were similar to those observed in fishes treated with the synthetic esterogen ethinylestradiol (EE2) (Schwaiger et al., 2000), ultraviolet radiation (Sayed et al., 2007; Mekkawy et al., 2010; Osman et al., 2010), pesticide (Adedeji et al., 2009) and heavy metals (Ololade and Oginni, 2010). Structural defects and changes in surface shape
of erythrocytes have been reported by Koc et al. (2008) from endosulfan (an endocrine disrupter) and malathion exposed rats. Changes in the erythrocyte profile were also noticed in fishes by Benarji and Rajedranath (1990) after dichlovos treatment. Similar trends of result were obtained by Travares et al. (1999) following trichlorphon exposure and Bushra et al. (2002) after 2, 4-dichlorophenoxyacetic acid and Butachlor exposure. BPA caused erythrocytic alterations similar to the works stated above.

Many studies have been undertaken to explain the probable causes of the change in alterations. The erythrocyte membrane skeleton is an organized network of structural proteins that interacts with the lipid bilayer and transmembrane proteins to maintain red cell morphology and mechanical stability. The major proteins of the membrane skeleton in vertebrates are α and β spectrin, actin, ankyrin and 4.1 protein (Branton et al., 1981; Liu et al., 1987). The erythroid specific protein 4.1 plays a crucial role in anchoring the spectrin–actin based cytoskeleton overlaying to the lipid of plasma membrane. Interactions between these proteins form a protein network and any qualitative or quantitative disruption of these protein–protein interactions may result in defective structure and function of red blood cell membrane (Shafizadeh et al., 2002). According to Tripathy and Srivastava (2010), chlorpyrifos caused alterations in the cytoskeleton (membrane protein and/or lipids) of red blood cells thus affecting the surface area of the cell. Comelekoglu et al. (2000) stated that some compounds may provoke the alterations in size and surface shapes of erythrocytes. Nikimma (1992) suggested that toxic materials directly or indirectly damage the membrane structure, ion permeability and cell metabolism of erythrocytes and thus may cause morphologically damaged erythrocyte formation. Thus, the abnormal cell morphology recorded from the erythrocytes of the test fishes of the present study could be attributed to the cytoskeleton fragility and defects in the structural proteins caused by BPA.

The fish erythrocytes have also shown to be transformed into various abnormal forms due to depressed adenosine triphosphate (ATP). Sawhney and Johal (2000) observed altered erythrocytes as a result of hypoxic condition induced by malathion. Some other reasons for transition of normal erythrocytes to altered ones induced the factors which
cause apoptosis of blood cells, like ionizing radiations, reactive oxygen intermediate inducing agent, exogenous oxidants, \textit{in vitro} ageing and increase in cytosolic calcium (Chukhlovin, 1996). Some of the cells showing plasmolysis or dissolution of cytoplasm and loss of cellular outlines could also presumably be undergoing necrosis. Mildly adverse conditions causing injury to the cells have shown to die by programmed cell death, while exposure to severe conditions leads directly to cell death by necrosis (Schwartzman and Cidlowski, 1993).

5.15. Presence of Estrogenic Heavy Metals in different water bodies around Guwahati City

Many of the sediments in our rivers, lakes and oceans have been contaminated by pollutants. Some of these pollutants are directly discharged by industrial plants and municipal sewage treatment plants, others come from polluted runoff in urban and agricultural areas, and some are the result of historical contamination. The inorganic minerals like sodium, potassium, calcium, magnesium and heavy metals like iron, manganese, lead, mercury, chromium, cadmium, nickel, cobalt, beryllium copper etc., when present above the permissible limit are harmful. Agricultural water pollution is caused by fertilizers, insecticides, pesticides, farm animal wastes and sediments. Research findings indicate that application and heavy doses of fertilizers pollutate ground water through leaching. Some heavy metals have been shown to have endocrine disrupting properties, interfering with the hypothalamic–pituitary–ovarian (HPO) axis. Many adverse reproductive effects related to heavy metals have been observed in both toxicological and epidemiological studies. The present investigation showed high levels of heavy metal contents in different water bodies around Guwahati city. The recorded values of all the metals analyzed for the present study were significantly far higher than the WHO’s recommended values (shown in Table 4.5, Chapter 4). These higher levels of heavy metal may also disrupt reproductive health as well as other physiological processes on exposed animal communities as well as human beings entering through the food chain.
5.15. Presence of Estrogenic Heavy Metals in different water bodies around Guwahati City

Roy, 2011

(Sepulveda et al., 1999). Reports suggested that, certain heavy metals such as mercury (Rurangwa et al., 1998), lead (Thomas, 1988), and cadmium (Thomas, 1989; Ruby et al., 2000; Kime et al., 1996) found to have toxic effects on reproductive physiology. Several heavy metals stimulate proliferation of MCF-7 human breast cancer cells (Martin et al., 2003; Brama et al., 2007; Choi et al., 2003; Martinez-Campa et al., 2006). Cadmium interacts with estrogen receptor-α (ER-α) (Martin et al., 2003; Brama et al., 2007), and binds to the ligand-binding domain of ER-α in cultured cells (Stoica et al., 2000). Cd stimulates estrogenic responses in vivo (Johnson et al., 2003; Alonso-Gonzalez et al., 2007) Ovariectomized rats injected with Cd had increased uterine weight, accelerated mammary gland growth/development, and accelerated vaginal opening (VO) (Johnson et al., 2003). Cd-induced estrogen-like responses were prevented by the antiestrogen ICI 182,780. Cd treatment also stimulated breast cancer cell proliferation by activating ER-α–dependent Akt (protein kinase B), Erk1/2 (extracellular signal-regulated kinase), and platelet-derived growth factor receptor-α (Brama et al., 2007). Similar to estradiol, treatment of cells with the divalent metals copper, cobalt, nickel, lead, mercury, tin, and chromium stimulated cell proliferation and there was a 2-5 fold increase in cell number. The metals also decreased the concentration of ER-α protein and mRNA by 40-60% and induced expression of the estrogen-regulated genes progesterone receptor and pS2 by 1.6-4 folds. The ability of the metals to alter gene expression was blocked by an antiestrogen, suggesting that the activity of these compounds is mediated by ER-α (Hogaboam et al., 2008).

Mercury appears to be responsible for feminization and reproduction problems of beluga whales and polar bears in the Arctic and panthers and alligators in Florida (Science News, Vol. 145 & 146, 1994; De Guise et al., 1994; Facemire, et al., 1995; Reiersen, et al., 2002) along with other effects and population declines of fish eating predators (Sepulveda et al., 1999). Levels of mercury in Florida were also sufficient to have contaminated lakes and bays in Florida to levels where fish in over half the lakes and streams tested have levels of mercury dangerous to wildlife or humans eating the fish. The same study showed that birds and
panthers in South Florida were dying as a result of mercury levels in the fish (Facemire et al., 1995; Sepulveda et al., 1999).

5.16. Gas Chromatography and Mass spectrometric (GC/MS) Analysis for Testing Estrogenic compounds

It is not surprising that rivers and lakes (beels) is loaded with several contaminants from domestic applications, industrial use and normal human practice. These chemicals are harmful for general health, for aquatic animals and plants. Several reports suggest the presence of a number of potential EDCs as well as toxicants in rivers across the globe and in finally treated sewage effluent. These chemicals include synthetic and natural androgens/estrogens, insecticides, pharmaceuticals, phenols, detergents etc (Ternes et al., 1999; Lucy et al., 2002, Thomas et al., 2002 and Ohte et al., 2006). The dramatic effects of endocrine disruption as well as toxicity has been shown in the aquatic animals exposed

The presence of EDCs in water samples is of great concern and is recognized as a potential problem. In the present study phthalates and pesticides were identified from three different sites around Guwahati city, Assam. EDC analysis of the water during the study period using GC/MS are presented in (Table 4.6, Chapter 4). The results showed the presence of estrogenic chemicals Aldrin, Dialdrin in Bharalu River (μg/l). The chemicals analyzed were below the detectable limit in Deepor beel, while Borsola beel recorded the presence of Dibutyl Phthalate (μg/l). The concentration of some of the other chemicals tested was generally below the limit of detection but was positively identified in three of the different water samples. Both phthalates and pesticides present above the permissible limits (WHO, 2003) pose a great threat to biodiversity and human sustenance. Similar studies were followed by (Brossa et al., 2002). They discovered
Pthalates (Dibutyl phthalate, benzylbutyl phthalate, bis phthalate and bis adipate together with other EDCs such as Atrazine, lindane, aldrin, dieldrin hexachloro benzene in water samples from Ebro river. Spain. Similarly (Pedersen and Linholst, 1999) quantified the presence of 4 tert octylphenol and BPA in water samples from Denmark. Estrogens (estradiol, estrone, estriol estradiol diacetate, ethynylestradiol), pesticides such as atrazine, simazine and plasticizer bisphenol A was detected from natural waters and drinking waters by (Rodriguez-Mozaz et al., 2004) in Barcelona, Spain. Thus our results corresponds and is in broad accordance to the studies of others.

The quality of water and sediment in the river system as well as the beels is seriously affected by pollutants which enter through drains that bring domestic as well as industrial effluents. These industrial and domestic waste waters, besides other pollutants also contain high concentration of EDCs. The presence of such compounds is a serious matter of concern because such EDCs enter human body through polluted waters which gets bioaccumulated in body’s muscle and tissues. Moreover by eating fishes from such polluted sites is also another route of exposure to such chemicals to human bodies, because EDCs get biomagnified in the food chain.